THE IMMUNOLOGICAL BASIS OF ACQUIRED CELLULAR RESISTANCE*

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Infection with certain organisms is often found to produce a high degree of resistance towards other, unrelated organisms (1). The phenomenon is conspicuous whenever the immunising infection is caused by a facultative intracellular parasite (2). The latter provokes an immune response with three peculiarities: a consistent association with delayed-type hypersensitivity, a form of acquired resistance that cannot be passively transferred with serum, and a reported change in the antibacterial activity of the host's macrophages.

It is usual to dismiss the cross-resistance caused by such organisms as an example of "non-specific immunity," and to ignore the possible immunological significance of the phenomenon. It was decided to reinvestigate the problem in the hope of arriving at a better understanding of the immunological processes involved in this type of acquired antibacterial resistance. In this report the specificity of both the *induction* and the *expression* of acquired resistance has been studied in mice immunised with three facultative intracellular parasites; *Listeria monocytogenes*, *Brucella abortus*, and *Mycobacterium tuberculosis*.

Materials and Methods

Mice.—The mice used were of an outbred strain (3). Animals of the one sex and of comparable age (7 to 8 weeks) were used in individual experiments.

Organisms.—The strain of *L. monocytogenes* (NCTC 7973, serotype I) was sustained at high virulence by continuous passage in normal mice. The attenuated vaccine strain of *Br. abortus* (strain 19) was obtained from the Commonwealth Serum Laboratories (Victoria). Both organisms were grown in brain-heart infusion broth (BHI, Difco Laboratories, Inc., Detroit). Suspensions of bacteria were prepared from 16-hour and 3-day cultures of *L. monocytogenes* and *Br. abortus*, respectively. The organisms were washed once and resuspended in Hanks' solution containing 0.1 per cent bovine serum albumin (fraction V, Armour). The LD_{50} for *L. monocytogenes* was 1.0×10^5 by intravenous injection; that for *Br. abortus* (strain 19) was not determined.

Bacterial Enumeration in the Spleen.—The method of determining the viable bacterial count in the spleen was previously described (3). Colony counts were made after 24 hours and 4 days for *Listeria* and *Brucella* cultures respectively.

Detection of Listeria-Resistant Macrophages.—The method used for preparing and parasitis-

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ing monolayers of mouse macrophages, and for washing them free of extracellular bacteria has already been described (3). The method for assessing the antibacterial activity of the cells, however, was modified in order to sample a larger proportion of the cells in a given population than was possible with the earlier plaquing techinque. Duplicate monolayers in open well-slides were maintained in culture for 24 hours at 37°C. They were washed twice before infecting them with a bacterial suspension containing 5×10^6 *Listeria*/ml of a culture medium consisting of 20 per cent foetal calf serum in Hanks' balanced salt solution. After 1 hour at 37°C the cultures were washed over a fountain with 200 ml of sterile saline, and one of them was fixed and stained to determine by direct microscopic count the number of bacteria present in the monolayers (3). The other was incubated for a further 5 hours in bacteria-free medium. This was then removed and the monolayer was left momentarily to dry before overlaying it with nutrient agar at 45°C. After a further 18 hours' incubation the cultures were illuminated with a darkfield condenser, and the numbers of bacterial microcolonies that had arisen were counted in a sample area, or over the entire culture if not too numerous.

Soluble Brucella Antigens.—A 4-day culture of *Br. abortus* (strain 19) grown in BHI medium was centrifuged to remove bacteria and sterilised by membrane filtration. The culture filtrate was used to test for hypersensitivity without further treatment.

Test for Delayed-Type Hypersensitivity.—Filtrate was injected in a volume of 50 μl into the right hindfoot-pad. The injection site was inspected after 6 to 8 hours to exclude an immediate reaction, and after 24 hours the thickness of each hindfoot was measured to 0.05 mm with dial-gauge calipers. The response was expressed as the percentage increase in thickness of the injected over the uninjected foot.

RESULTS

The Bacterial Growth Pattern and Host Response of Mice Infected with Br. abortus (Strain 19).—It was first necessary to determine the growth curve of *Br. abortus* in the mouse spleen and to deduce from this the time course of the host's response to the infection. In addition, the onset and the level of delayed-type hypersensitivity developed towards *Brucella* antigens was measured by foot-pad tests performed at intervals during the infection.

Mice were infected with *Br. abortus* by intravenous injection of approximately 5×10^6 organisms. Twenty-four hours later and then at 4-day intervals, 5 mice were used to determine the content of viable organisms in whole spleen homogenates. At 2-day intervals foot-pad tests were performed on 5 mice injected with 0.05 ml of a sterile culture filtrate of *Br. abortus*. The residual thickening at 24 hours in 5 normal (uninfected) mice injected with the same dose of culture filtrate was subtracted from the mean increase in foot-pad thickness found in the mice of the infected group. In no case did the residual foot-pad thickening of control mice exceed 5.5 per cent (range 3.7 to 5.5). Mice were used only once in tests for hypersensitivity.

Fig. 1 shows the growth pattern of *Br. abortus* in the spleens of normal mice, and the corresponding level of hypersensitivity found at each stage of the infection. The spleen counts indicate that the organism multiplied slowly for the first 9 days. Multiplication then ceased and the *Brucella* population underwent partial inactivation. This phase was followed by a period of latency in which the spleen count remained relatively constant. The spleen counts showed surprisingly little variation among mice at any stage. Much more variation was found in a *Listeria* infection (3).

A typical delayed-type hypersensitivity developed. It was first evident on the 5th day and increased rapidly for several days. Sensitivity then diminished to relatively low levels as the bacterial population approached its peak. During the period of bacterial inactivation it rose again and remained high throughout the period of observation.

The Growth of L. monocytogenes in the Spleens of Mice Infected with Br. abortus (Strain 19).—It appeared from the foregoing results that an infection of mice with this strain of *Br. abortus* is divisible into three distinct phases. It was de-

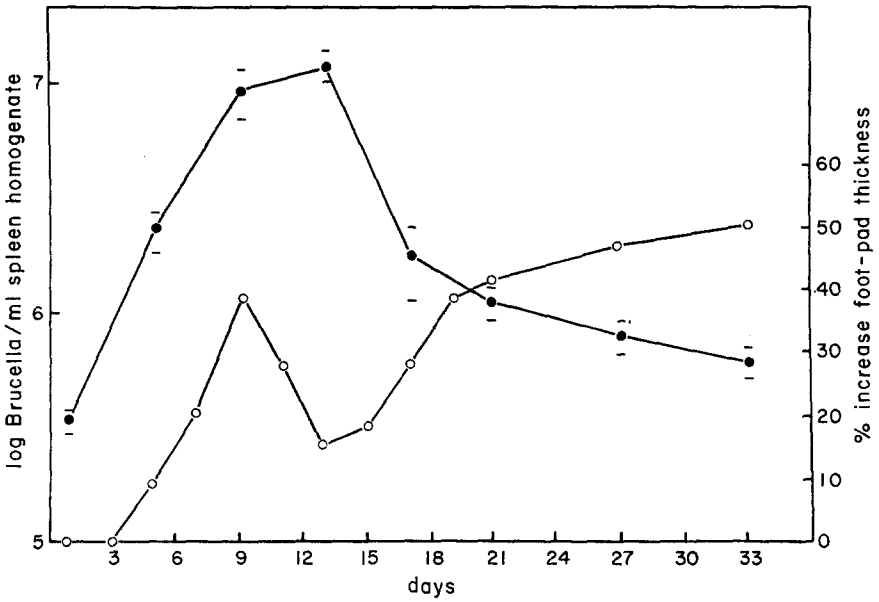

FIG. 1. Mean viable count and standard deviation in the spleen (●——●), and the corresponding level of delayed-type hypersensitivity to *Brucella* antigens (○——○) found at intervals during a primary *Br. abortus* infection in normal mice. Five mice per group.

cided to test for resistance to *L. monocytogenes* at each phase, and at the same time to examine the peritoneal macrophages of the *Brucella*-infected animals for their ability to inactivate *L. monocytogenes in vitro*. A smaller dose of *Brucella* was used in the hope that each phase of infection would be more protracted.

A large group of mice was injected intravenously with a dose of 5.6×10^6 viable *Br. abortus*. Some of the animals were used for establishing the *Brucella* curve in the spleen, and the remainder, together with an equal number of uninfected control mice, were divided into groups of 36. On the, 4th 18th, and 35th days of the *Brucella* infection the mice of one group, and their controls, were challenged intravenously with *L. monocytogenes* (approximate dose, 0.5 LD_{50}). Following each challenge the growth curve of *Listeria* was determined from viable

counts made on the spleens of 6 normal and 6 *Brucella*-infected animals killed on successive days. Monolayer cultures were prepared from the peritoneal washings taken from mice killed 12 hours after the *Listeria* challenge. These were tested for anti-*Listeria* activity in the manner described under Materials and Methods.

It can be seen from the upper part of Fig. 2 that the growth of *Br. abortus* in the spleen followed a pattern similar to that of the previous experiment in

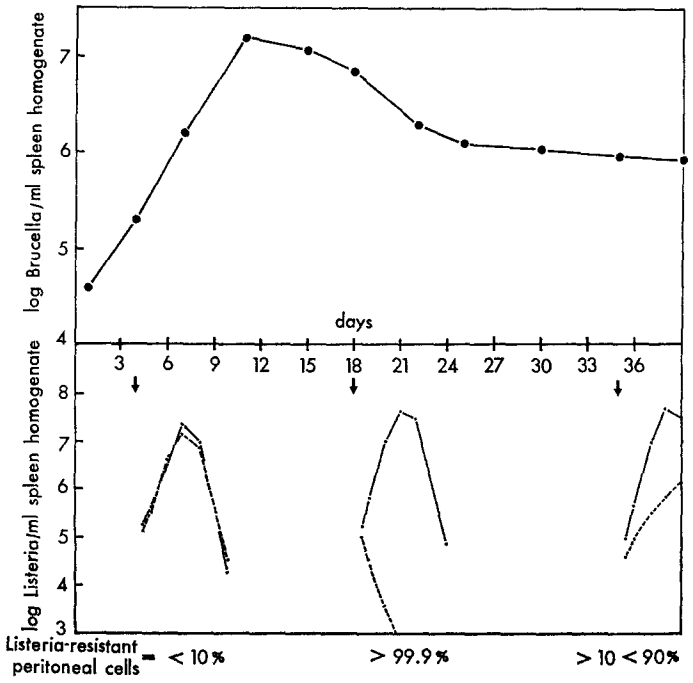

FIG. 2. *Top:* Growth curve of *Br. abortus* in the spleens of normal mice. Means of 5 mice per group. *Bottom:* Growth curves of a challenge inoculum of *L. monocytogenes*, injected at stages (arrowed) during the course of *Brucella* infection shown above. ●——●, normal controls; ●----●, *Brucella*-infected mice. Means of 6 mice per group.

The content of *Listeria*-resistant macrophages in the peritoneal cavity at the time of challenge is shown below the figure (see also Table I).

which these phases were described. The three inocula of *L. monocytogenes* that were injected on days 4, 18, and 35 behaved in a manner that was characteristic of the phase of the *Brucella* infection at that time. This is depicted in the lower half of Fig. 2. When injected on the 4th day the *Listeria* went through a normal growth cycle in the spleen. Those injected on the 18th day, however, were rapidly inactivated. Indeed the rate of inactivation exceeded that seen at the height of an immune response to *L. monocytogenes* itself (3). By the 35th day,

the *Listeria* were again able to multiply in the *Brucella*-infected spleen, but the rate of growth was somewhat reduced.

The marked differences in the behaviour of *L. monocytogenes* in the spleen at successive stages of the *Brucella* infection were faithfully reflected in the macrophage populations recovered from the peritoneal cavity at the corresponding times. This is shown in the data of Table 1 which sets out the numbers of microcolonies which arose in monolayers of *Listeria*-infected macrophages obtained from the normal or *Brucella*-infected mice. It will be seen that more than 99.9 per cent of the bacteria present in the cultures from the *Brucella*-infected animals on the 18th day had been inactivated. Direct counts consistently showed, moreover, that the cultures of immune cells prepared at this

TABLE I

Numbers of Microcolonies Arising in Monolayers of Listeria-Infected Macrophages Obtained from Normal or Brucella-Infected Mice

Mouse	*Brucella* infection					
	Day 4		Day 18		Day 35	
	Normal	*Brucella*-infected	Normal	*Brucella*-infected	Normal	*Brucella*-infected
1	1.9×10^4	2.1×10^4	0.9×10^4	7	0.8×10^4	1.2×10^3
2	1.1×10^4	1.6×10^4	1.9×10^4	17	1.1×10^4	1.3×10^3
3	2.8×10^4	1.9×10^4	2.8×10^4	40	0.9×10^4	1.6×10^2
4	1.0×10^4	0.8×10^4	1.1×10^4	24	1.9×10^4	2.9×10^3
5	0.8×10^4	0.9×10^4	1.9×10^4	15	1.2×10^4	1.8×10^2
6	3.6×10^4	2.5×10^4	2.6×10^4	10	1.0×10^4	1.6×10^3
Mean...	1.9×10^4	1.6×10^4	1.9×10^4	17	1.2×10^4	1.2×10^3

time contained more intracellular *Listeria* than were present in the control cultures. This capacity of the macrophages to inactivate *Listeria in vitro* had diminished considerably in the monolayers prepared from the *Brucella*-infected mice on the 35th day of infection.

The Growth of Br. abortus (Strain 19) in the Spleens of Listeria-Immunised Mice.—It was not possible to reverse the plan of the previous experiment for the purpose of studying the behaviour of *Br. abortus* in the spleens of *Listeria*-immune mice. Not only are the time relations quite different, but there is also the difficulty that immunisation with *L. monocytogenes* necessitates the use of living organisms. This meant that mice could not be challenged with *Br. abortus* earlier than the 7th day of the immunising *Listeria* infection, because the *Listeria*, being more numerous and faster growing than *Brucella*, would have obscured the plates.

Mice were injected intravenously with a sublethal dose of *L. monocytogenes* (0.1 LD_{50}). Seven days later they were challenged, together with a group of unimmunised controls, by the intravenous injection of living *Br. abortus*. At intervals the spleens of 6 mice were homogenised for enumeration of their content of viable *Brucella*.

Fig. 3 shows the growth curves of *Br. abortus* in the spleens of normal mice and of mice infected with *L. monocytogenes* 1 week previously. The numbers of viable *Brucella* present in the spleens of the *Listeria*-immunised mice were

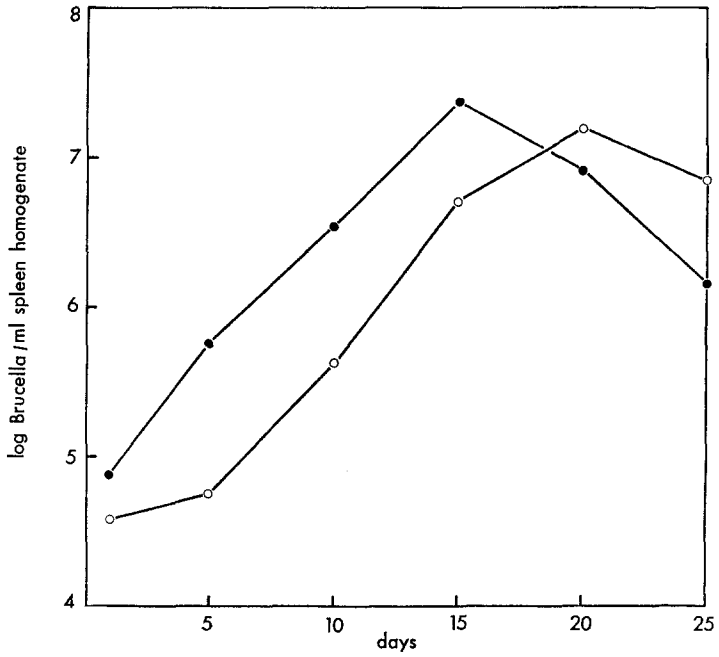

FIG. 3. Growth curves of *Br. abortus* in the spleens of normal mice (●——●) and mice infected 7 days previously with a sublethal dose of *L. monocytogenes* (○——○). Means of 6 mice per group.

fewer at 24 hours than in controls, and showed little evidence of increase during the next 5 days. Beyond the 1st week they grew at an equal rate in normal and immunised animals. Apparently the antecedent *Listeria* infection had created in the spleen a short-lived change affecting the growth and survival of *Br. abortus*. The finding that this effect did not persist longer than 2 weeks from the initiation of the *Listeria* infection is consistent with a previous observation that the period of absolute resistance to reinfection of the spleen with *Listeria* itself is of similar duration. It corresponds to the time during which the peritoneal macrophages were found to be resistant to *Listeria in vitro* (3). Later in the convalescence of *Listeria*-infected mice the reinjection of *L. monocyto-*

genes produces a rapid reactivation of the immune mechanism. This being so, it seemed possible that a sustained level of resistance to *Br. abortus* in *Listeria*-immunised animals could be produced by periodic reinfection with *L. monocytogenes*.

Mice were infected intravenously with approximately 10^6 *Br. abortus*. Twenty-four hours later half of them were injected intravenously with a small (0.01 LD_{50}) immunising dose of *L. monocytogenes*. Those remaining unused on the 5th and 9th days were given a larger (10 LD_{50}) reinfecting dose of *L. monocytogenes* by the same route. The growth of *Br. abortus* in the spleens of the untreated and *Listeria*-immunised mice was determined in the usual way.

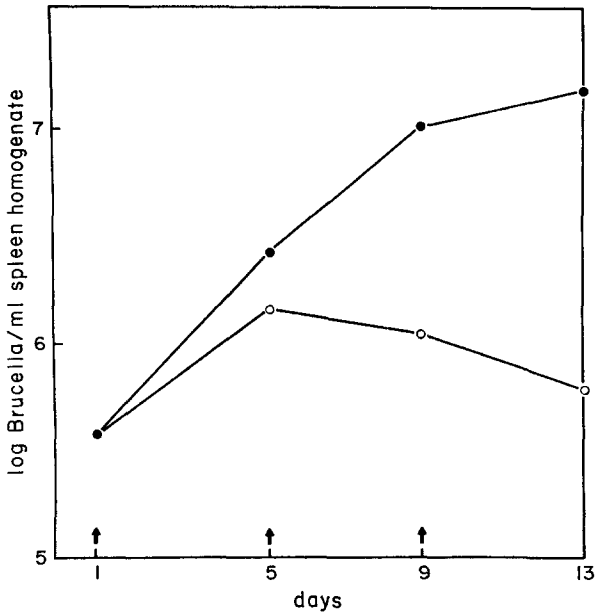

FIG. 4. Growth and survival curves of *Br. abortus* in the spleens of normal mice (●——●) and of mice infected on day 1 (0.01 LD_{50}), and reinfected on days 5 and 9 (10.0 LD_{50}) with living *L. monocytogenes* (○——○). Means of five mice per group.

Fig. 4 compares the growth pattern of *Br. abortus* in the spleens of untreated mice and of mice repeatedly reinfected with *L. monocytogenes* during the course of a primary *Brucella* infection. It is apparent that the *Listeria* infection caused a very effective and persisting inhibition of the growth of *Br. abortus*. The resistance to *Brucella* appears to have been due entirely to the effects produced by the *Listeria* infection, for it had commenced by the 4th day (3), and was well established long before the untreated mice showed evidence of the onset of resistance induced by *Brucella* itself.

The Specificity of the Recall of Acquired Resistance to L. monocytogenes and

Br. abortus.—It will be noted from Fig. 3 that the inhibition of *Br. abortus* was short-lived in animals immunised with a single dose of *L. monocytogenes*. Beyond the 5th day the challenge organism grew as rapidly in immunised animals as it did in normal controls. This seemed to indicate that the recall of acquired resistance involves a mechanism that is immunologically specific. This question was examined in both *Listeria*- and *Brucella*-sensitised animals.

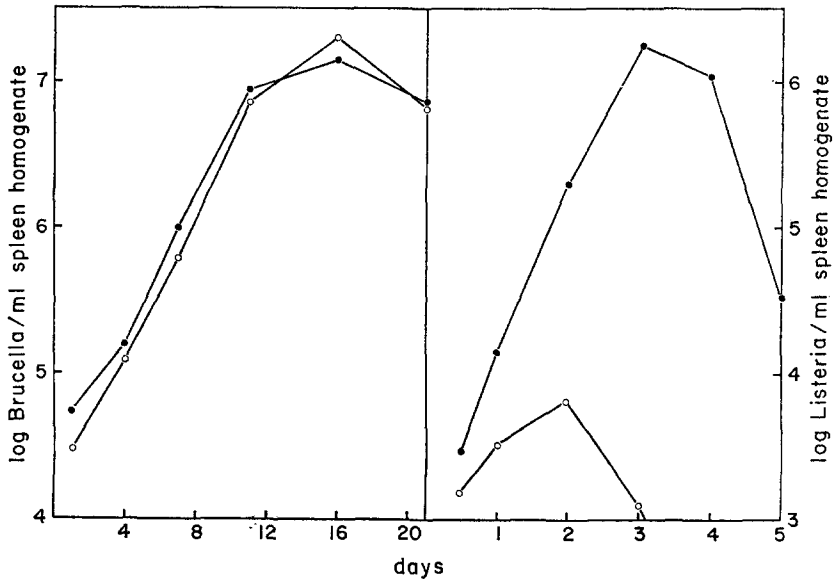

FIG. 5. Growth and survival curves of *Br. abortus* (left) and *L. monocytogenes* (right) in the spleens of normal mice (●——●) and mice immunised 4 weeks previously with a sublethal dose of *L. monocytogenes* (○——○). Means of 6 mice per group.

Listeria-Sensitised Mice.—

Mice were injected intravenously with a primary immunising dose of *L. monocytogenes* (0.1 LD_{50}). Four weeks later half of them were challenged intravenously with virulent *Listeria* (1.0 LD_{50}), and the other half with *Br. abortus* (1.6×10^6). Two other groups of control mice were challenged in the same way at the same time. Viable counts were performed at intervals on the spleens of 6 mice from each group.

Fig. 5 shows a comparison between the growth of *Br. abortus* and *L. monocytogenes* in the spleens of normal mice and mice which had been immunised 4 weeks previously with *L. monocytogenes*. Whereas the challenging dose of *Listeria* evoked an accelerated immune response in the tissues of *Listeria*-sensitised mice, *Br. abortus* failed to do so. Reference to Fig. 2 will show that *L. monocytogenes* injected during the latent phase (day 35) of a *Brucella* in-

fection grew slowly but without interruption for at least 4 days, indicating again that heterologous organisms are incapable of reactivating the antibacterial mechanism of sensitised mice.

Brucella-Sensitised Mice.—Use was made of the fact that mice infected with *Br. abortus* (strain 19) pass into a latent phase in which viable organisms persist in the tissues in relatively small numbers (Fig. 2). In view of the foregoing results it would be expected that reinfection of such animals with homologous organisms would reactivate the host's bactericidal mechanism and disturb the established balance between the host and the original parasite population.

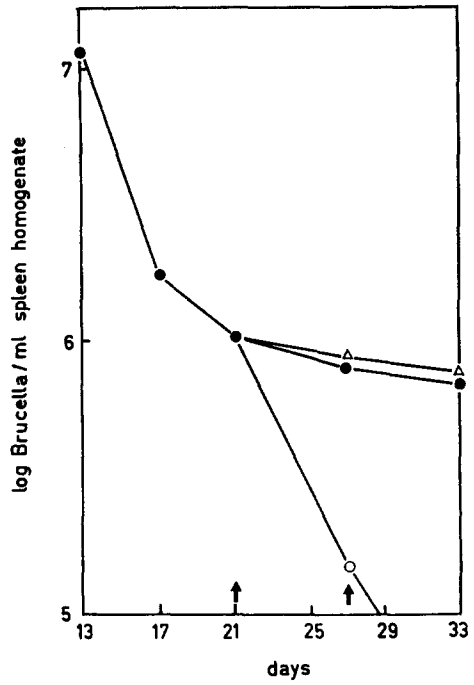

FIG. 6. Bacterial inactivation curves in the spleen during the 3 to 5 week interval following a primary *Brucella* infection in normal mice (●——●) and in mice reinfected on days 21 and 27 (arrowed) by the intraperitoneal injection of 10^8 living *Br. abortus* (○——○), or BCG (△——△). Means of 5 mice per group.

Mice were infected intravenously with about 10^6 *Br. abortus* (strain 19). Spleen counts on groups of 5 mice were made at intervals beginning on day 13 of the infection. On days 21 and 27 some of the mice were injected intraperitoneally with BCG or *Br. abortus* in a dose of 10^8 organisms. The remainder was left untreated. The numbers of viable *Brucella* found in the spleens of the reinfected and unchallenged mice are shown in Fig. 6.

The peritoneal route of reinfection was chosen in order to minimise the number of new organisms entering the spleen. It was hoped that in this way it

would be possible to study the fate of the original spleen population of *Brucella*. Despite the certainty that a number of organisms from the reinfecting inoculum entered the spleen, the viable *Brucella* count fell in the spleens of mice reinfected with *Br. abortus*. It did not change in mice challenged with BCG, indicating again the specificity involved in the process of activation of the antibacterial mechanism.

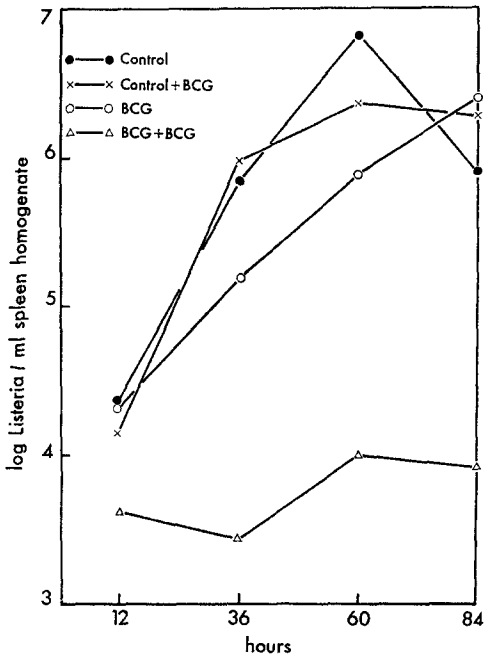

FIG. 7. Growth curves of *L. monocytogenes* in the spleens of normal or BCG-sensitised mice given an intravenous injection of BCG 3 days prior to challenge with *L. monocytogenes*. ●——●, normal mice; ○——○, BCG-sensitised mice; ×——×, normal mice given BCG prior to challenge; △——△, BCG-sensitised mice given BCG prior to challenge. Means of 6 mice per group.

The Effect of Antigen on the Level of Acquired Cellular Resistance.—It could be argued from the evidence of Figs. 1 and 2 that the only period during which an effective bactericidal mechanism was present in the spleens and the peritoneal macrophages of *Brucella*-infected mice occurred in the period immediately following the peak concentration of antigenic material in the tissues. A comparison of Figs. 3 and 4 also indicates that persistence of antigen in the tissues of *Listeria*-immunised animals is a necessary condition for the expression of resistance against *Br. abortus*. This raises the interesting question of whether the bacterial antigens are themselves playing a critical role in the induction of

acquired cellular resistance. In order to investigate this point, and to add further evidence on the question of specificity, a study was made of the effect of a sudden increment in the antigen content of the tissues of BCG-sensitised mice.

Mice were injected intravenously with 0.25 mg (moist weight) of a vaccine strain of BCG. Half of the sensitised animals, and an equal number of normal mice, were given a second intravenous injection of BCG 14 weeks later when their spleens still contained viable tubercle bacilli. All mice, together with an additional group of untreated controls were challenged intravenously with *L. monocytogenes* (1.0 LD_{50}) 3 days following the second injection of BCG. The numbers of viable *Listeria* in the spleens of 5 mice from each of the four groups were determined at daily intervals.

Fig. 7 shows the growth curves of *L. monocytogenes* in the spleens of mice of the four treatment groups of the foregoing experiment. The curves show three significant features. (*a*) The rate of growth of *Listeria* was diminished in BCG-immunised mice and was comparable with that seen in the latent phase of a *Brucella* infection (Fig. 2). (*b*) There was no evidence of an accelerated immune response to *Listeria* in BCG-sensitised animals. (*c*) A marked effect on survival and growth of *Listeria* occurred when BCG was injected into BCG-sensitised animals, but not when injected into normal mice.

DISCUSSION

The antibacterial mechanism which develops in mice infected with *L. monocytogenes*, *Br. abortus*, or *M. tuberculosis* is clearly not directed exclusively against the organism which provoked it. It is, in fact, quite non-specific. The extreme antigenic diversity among the bacterial species which promote a mutual cross-resistance makes it unlikely that the mediator is an antibody which modifies the bacterial cell by direct union with it, as in opsonisation or bacteriolysis. The experiments in the preceding paper (4) support this view. They failed to show evidence of any free or cell-bound antibody in *Listeria*-immune mice that could significantly influence the survival and growth of *L. monocytogenes* in a wide variety of test systems.

On the other hand previous studies (3–5), and some of the present observations, have shown that the macrophages of immunised animals acquire antibacterial properties that are not found in the cells of normal animals. Since acquired resistance may sometimes be due entirely to this change in cellular activity, it is important to enquire into the mechanism of its induction.

The present studies have shown that the underlying events which lead to altered functional activity in host macrophages are immunologically determined and specific in nature. The evidence depends upon the finding that reinfection with homologous organisms effectively recalls the state of acquired resistance, while challenge with heterologous organisms does not. This was true of all cases that could be tested conveniently. It can be concluded, therefore, that infection produces a state of immunological reactivity which is susceptible to activation only by the microbial antigens to which the host has

been rendered sensitive. For the reasons already given it is unlikely that this state of reactivity is merely a capacity to respond anamnestically by an accelerated production of antibody; for this would not explain the absence of specificity in the ultimate mechanism of bacterial inactivation. For instance, the reinjection of BCG into BCG-sensitised animals produced a marked degree of resistance to *L. monocytogenes*; the reinjection of *L. monocytogenes* into *Listeria*-sensitised animals produced a sustained resistance to *Br. abortus*, and the injection of *Br. abortus* into *Brucella*-sensitised mice produced macrophages with anti-*Listeria* activity. It seems, therefore, that non-specific resistance reappears, or is augmented, soon after the introduction of the specific microbial antigens to which the tissues are already sensitive. If this is true, it implies that both antigen and specific sensitisation of the tissues are necessary factors for the induction of this form of acquired resistance.

The nature of the background of sensitisation which appears to be essential for the induction of acquired cellular resistance has not yet been identified with certainty. It must be considered highly significant, however, that all the organisms used in the present study provoke an immune response attended by delayed-type hypersensitivity. In view of the absolute specificity of the *recall* of acquired resistance and the known specificity of delayed hypersensitivity to *Brucella*, *Listeria*, and mycobacterial antigens, it is worth discussing the possibility that both these immunological phenomena are mediated by the same antibody.

Delayed-type hypersensitivity to the respective soluble antigens of *L. monocytogenes* (3) or *Br. abortus* (Fig. 1) occurred in each case on about the 4th day of a primary infection. In the former the onset of the antibacterial mechanism also occurred on the 4th day, but in brucellosis it was delayed for a further 5 or 6 days. *Br. abortus* is a much more slowly growing organism, and is more resistant to inactivation (Fig. 2). If release of antigen into a hypersensitive host is an essential step in the induction of acquired resistance, it would be expected that the antibacterial mechanism would emerge more slowly in a *Brucella* than in a *Listeria* infection.

In the present context it is significant that the level of hypersensitivity to *Brucella* antigens fell as the *Brucella* population approached its peak (Fig. 1). This can be taken as an indication that antigen was being released in amounts sufficient to cause partial desensitisation of the host. It was during this period that the antibacterial mechanism was most efficient. The coincident onset of hypersensitivity and resistance in a *Listeria* infection and the desensitisation that occurred at the time of active bacterial destruction in a *Brucella* infection both point to a significant relationship between hypersensitivity and acquired cellular resistance. It is clear, however, that the antibacterial mechanism is not due to hypersensitivity alone, for hypersensitivity was present in higher degree both early and late in the infection when resistance was absent or low. But

at these times the bacterial population was small, and the extent of the allergic reaction occurring in the tissues would be correspondingly reduced. If this explanation is correct, it follows that the introduction of additional antigen during a latent infection, or during convalescence in the case of listeriosis, should be accompanied by a prompt increase in the level of host resistance. This was shown to occur in each of the three infections used in the present investigation (Figs. 4, 6, and 7).

Some of the features of a tuberculous infection are consistent with the foregoing hypothesis that acquired cellular resistance depends upon antigenic activation of tissues sensitised to specific microbial antigens. The protective effects of vaccination with BCG can be revealed in a number of ways: by a difference in survival time of the host, a reduction in the number of virulent organisms in the tissues at an interval following challenge, or by a difference in the severity of lesions. However, when quantitative studies were made by Lévy *et al.* (6), it was found that similar numbers of viable organisms could be recovered from normal and vaccinated animals for some time following challenge, and that equivalent numbers of microscopic lesions developed in relation to them. Evidently the benefits of vaccination were not immediately available to the host, but were invoked by the challenge organism itself. In terms of the present hypothesis, the delay in the onset of resistance is explained by the time needed for the virulent organism to increase in numbers to a point at which the amount of antigen being released into the tissues is sufficient to activate the antibacterial mechanism of host macrophages.

At present we know very little of the nature of the antibody that confers delayed-type hypersensitivity, the highly specific nature of which demands the existence of patterned molecules that are capable of reacting with the determinant groups of the corresponding antigens. Boyden's recent studies (7) have suggested that delayed-type hypersensitivity may be mediated by a cytophilic antibody which possesses a strong affinity for the surface of macrophages. If the antigen-antibody reaction which is postulated to form the basis of acquired cellular resistance be true, no better location for the hypothetical antibody could be imagined than on the surface of the macrophage itself. The interaction of antigen and antibody would then take place on the very cells which are now known to undergo changes during infection. Apart from their striking antibacterial properties, the macrophages of immunised animals show gross differences in morphology (3) and minor differences in ultrastructure (8). They show metabolic activity in excess of that found in normal cells (9) and cytochemical evidence of altered metabolic habit (10). The likelihood that some of these changes are due to a stimulating effect of antigens on sensitised cells is evident from the studies of Waksman and Matoltsy (11), and from the fact that the macrophages of animals allergically sensitised to soluble antigens respond with intense mitotic activity when the corresponding antigen is in-

jected (12). Evidence will be presented in a forthcoming publication that the sensitised host responds to bacterial antigens in exactly the same way (13). The fact that the macrophages of sensitised animals respond to specific antigen by DNA synthesis and subsequent division is of potential significance in relation to the mechanism of acquired cellular resistance. This is true whether the response of macrophages is due to a direct effect of antigen on cells sensitised by adsorbed antibody, or whether it is due to a more complex train of events.

The proposition that the mediator of delayed-type hypersensitivity provides the immunological reactivity upon which acquired cellular resistance depends will be criticised by those who believe that hypersensitivity and acquired resistance are separable phenomena (14, 15). Evidence for the latter view usually takes one of two forms: the finding that resistance tends to persist in animals which have been desensitised by the repeated injection of antigen (16); or that appropriate bacterial extracts can cause increased resistance without producing cutaneous sensitivity (17) or cutaneous sensitivity without increased resistance (18). None of this evidence is in conflict with the view that resistance depends for its full expression upon the presence of hypersensitivity. Although the injection of antigen may eliminate cutaneous sensitivity (a small single injection of culture filtrate will completely desensitise *Listeria*-sensitised mice for several days, reference 19), it does not necessarily eliminate the activated macrophages upon which resistance depends. Indeed, according to the present evidence it would tend to promote them to increased functional activity. The production of resistance without hypersensitivity may merely mean that the immunising antigen has produced a primary antibody response of very small proportions, but sufficient to ensure a rapid anamnestic response on challenge and the consequent background of hypersensitivity upon which resistance depends. The production of sensitivity in the absence of increased resistance, for instance by the injection of PPD and bacillary wax (18), is more difficult to explain. We should remember, however, that such procedures invariably produce a precipitating or Arthus-type of antibody in addition to the antibody which mediates delayed-type hypersensitivity (20, 21). It is possible that circulating antibody would seriously interfere with the immune mechanism by combining with free antigen, and thus reduce its effective concentration so far as the reaction with sensitised macrophages is concerned. The case of graft enhancement is perhaps analogous (22). It should be remembered, too, that when tuberculosis is the subject of study a very long time elapses before the results of challenge are known. This leaves time in which all manner of change can occur in the level of hypersensitivity and in the concentration of microbial antigens in the tissues. As we have seen in the case of a *Brucella* infection, host resistance and cutaneous sensitivity are subject to wide fluctuation. They seem to vary inversely with the prevailing size of the parasite population. There is clearly a dynamic interaction going on that requires a much more detailed analysis.

The foregoing speculations on the nature of acquired cellular resistance were prompted by the important consideration that out of a specific immunological reaction, a non-specific antibacterial mechanism emerges. It is unlikely, therefore, that bacterial inactivation is the consequence of an antigen-antibody reaction taking place on the bacterial cell, for this would not explain the host's behaviour towards heterologous organisms. It seems more likely that the reaction takes place between antigen and a host component which has become sensitised by the adsorption of specific antibody. The macrophage surface seems to provide an ideal site for this proposed antigen-antibody reaction to occur. This interpretation of acquired cellular resistance has certain merits, for it explains why resistance tends to be short-lived in those infections in which the antibacterial mechanism depends for its full expression upon the continuing presence of antigen, as in the well known instances of infection immunity. It also explains the non-specific nature of this type of acquired resistance, because it involves modification of the host rather than the parasite, and it leads to a better understanding of why an infection tends to become latent as the antigenic stimulus from a diminishing microbial population gradually subsides.

SUMMARY

The resistance developed by mice during infection with *Listeria monocytogenes*, *Brucella abortus*, or *Mycobacterium tuberculosis* is not specifically directed against the infecting organism. The processes involved in the induction of acquired resistance, however, are highly specific and seem to depend upon two factors: a state of immunological reactivity of the host and the presence of the specific microbial antigens to which the host has become reactive. When these two coexist in the tissues the host is found to be non-specifically resistant. It is suggested that resistance, which was shown to depend upon an altered state of host macrophages, may be due to the interaction of antigen and a specific antibody adsorbed to the surface of host macrophages; and that the antibody involved in the reaction is perhaps identical with the antibody which confers the state of delayed-type hypersensitivity. The results are discussed in relation to the question of latent infection and infection immunity.

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